ΜΕΤΡΗΣΗ ΠΡΩΤΕΪΝΩΝ ΜΕ ΤΗΝ ΜΕΘΟΔΟ MULTIPLEX ELISA

Εμβιομηχανική και Βιοϊατρική Τεχνολογία
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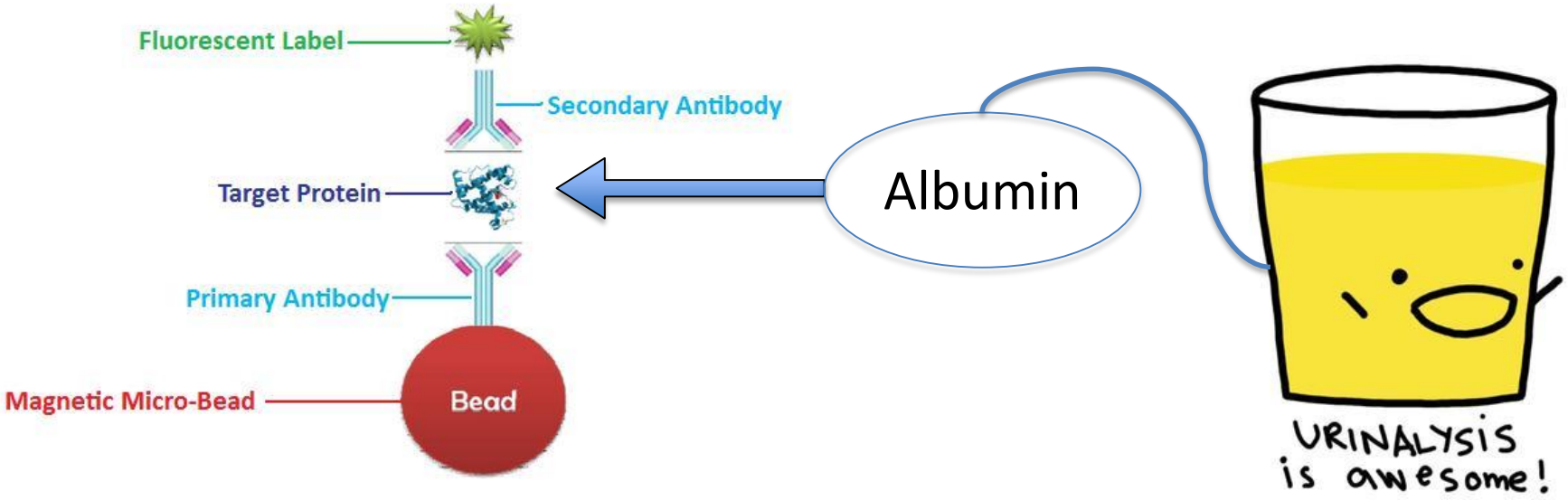
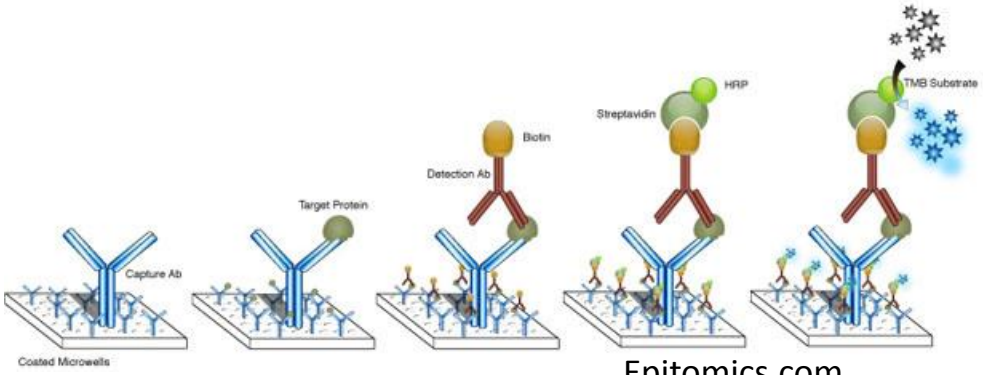
Lab Objective

- Utilize the bead-based multiplex ELISA method to quantify the concentration of Albumin protein in urine samples

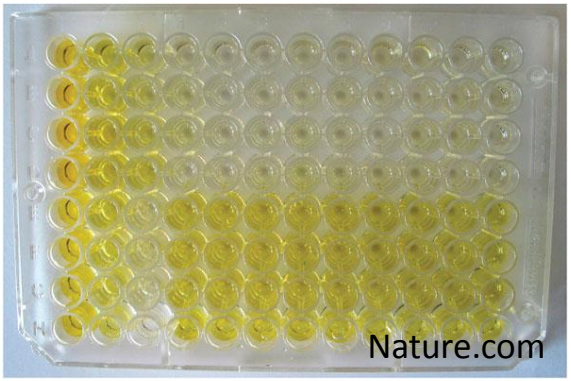


Intro: ELISA

- A standard biochemical method for quantifying the concentration of a protein in solution
  - Utilize 2 antibodies (capture ab, detection ab)



Epitomics.com



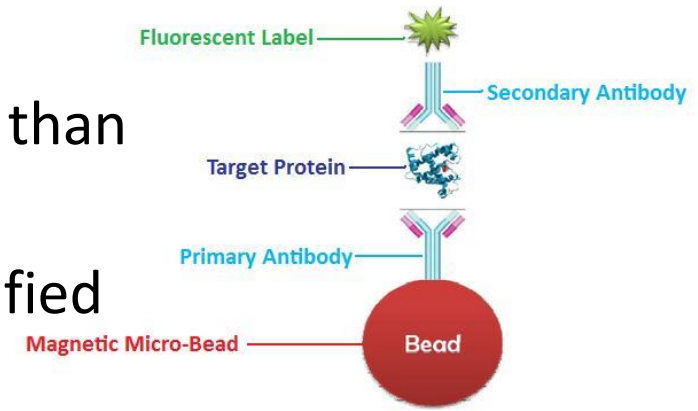
  - Takes place in 96-well plates, where caption ab is immobilized
  - Quantifies 1 protein per solution
  - Utilizes a standard curve to calibrate detected signal

Albumin

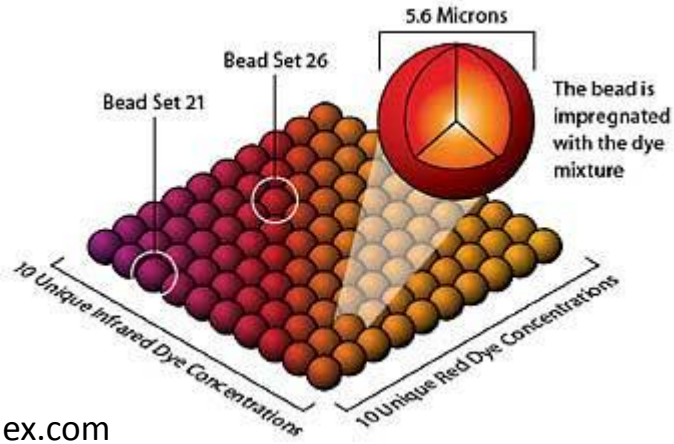
- Main component protein of human blood plasma
- Regulates the pressure of blood
- albuminuria: having too much albumin in the urine, if persistent it is linked to Chronic Kidney Disease (CKD)
  - 30 to 300 mg of albumin means albuminuria

Bead-based Multiplex ELISA

- Bead based assay
  - Immobilize capture ab on beads rather than well walls
  - Then add beads to sample to be quantified



- Multiplex assay
  - A type of assay that simultaneously measures multiple analytes (proteins) in a single run.
  - Here: add to each sample several kinds o beads, each kind can quantify a different protein
  - Luminex xMAP technology is one such example



Luminex.com

xMAP Multiplex ELISA Technology

1. Color-coded beads, pre-coated with analyte-specific capture antibody for the molecule of interest are added.
2. Analyte-specific antibodies capture the analyte of interest.
3. Biotinylated detection antibodies specific to the analyte of interest are added and form an antibody-antigen sandwich.
4. Phycoerythrin (PE)-conjugated Streptavidin is added.
5. The beads are read by a dual-laser flow-based detection instrument which excites the internal dyes marking the beads set and a second laser excites PE, the fluorescent dye on the reporter molecule.

- The system is capable of measuring potentially up to 100 analytes simultaneously in a small sample volume (25–50 µL).

Advantages of Multiplex ELISA

- **Speed/High Throughput:** each microsphere serves as an individual test, thus a large number of different assays can be performed and analyzed simultaneously.

- **Accuracy:** generates real-time analysis and accurate quantification of the biological interactions, limited sample handling, and decreased time and cost.

Standard vs Multiplex Bead-Based ELISA

- Standard ELISA
  - Quantify just one analyte at a time → slow, low throughput
  - Widely utilized all over the world
- MBAA techniques
  - Can be multiplexed → can quantify multiple proteins per sample → high throughput
  - May suffer from antibody cross-reactivity that causes distorted results
    - Only high-quality antibodies can be used
  - Require specialized instruments